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Interindividual Variability in the Metabolism and Cardiovascular Effects of Nicotine in Man[1]

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ABSTRACT

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We infused nicotine at rates of 1.0 to 2.0 μg/kg/min for 30 min into 14 healthy young men to determine individual differences in metabolic and renal clearance of nicotine and the relationship between cardiovascular effects and plasma concentration of nicotine. The mean (±S.D.) metabolic clearance was 1089 ± 279 ml/min; mean renal clearance (in acid urine) was 203 ± 58 ml/min, averaging 15.7% of total clearance. Steady-state volume of distribution was large (mean 183 ± 49 l). The large volume of distribution, despite a relatively high clearance, accounts for a terminal half-life which is relatively long, 119 ± 44 min. Infusion of nicotine increased heart rate and blood pressure and decreased fingertip skin temperature in a manner similar to cigarette smoking. Heart rate promptly increased, reaching a maximum within 5 or 10 min, despite progressively increasing plasma nicotine concentrations. After infusion, heart rate effects were less for a given nicotine concentration compared to during infusion, consistent with the development of tolerance. In contrast, skin temperature declined gradually during nicotine infusion and returned to base line after infusion in direct relationship to plasma nicotine concentrations with no evidence of tolerance. The substantial interindividual differences in nicotine metabolism may be a factor in determining cigarette smoking. Sensitivity of heart rate acceleration to low concentrations of nicotine and rapid development of tolerance to higher concentrations suggests that this and perhaps other nicotine-mediated cardiac responses may not differ when smoking low compared to high nicotine cigarettes. However, the decline in skin temperature, reflecting cutaneous blood flow, was related to blood nicotine concentration. The magnitude of nicotine-mediated vasoconstrictor responses may reflect amount of nicotine consumed and the time course of response reflect the time course of persistence of nicotine in the body.

Some cigarette smokers regulate nicotine intake to maintain relatively constant body levels of nicotine (Russell, 1976; Lader, 1978). Individual differences in nicotine metabolism might be important in influencing smoking behavior. For example, to maintain body nicotine concentrations comparable to other smokers, persons who metabolize nicotine rapidly may inhale more smoke with greater intake of toxic combustion products. The present study examines interindividual differences in metabolic and renal clearance of nicotine.

Nicotine activates the sympathoadrenal system, increasing heart rate, contractility, vascular resistance, blood pressure and circulating catecholamines (Ball and Turner, 1974; Cryer *et al.*, 1977). Little is known about the relationship between nicotine dose or blood concentration and cardiovascular effects. Because understanding this dose-response relationship is important in designing safer cigarettes, cardiovascular consequences and pharmacodynamics of nicotine were also examined.

Methods

Fourteen healthy men, 21 to 34 years of age, who were regular cigarette smokers were the subjects. They smoked an average of 18.4 cigarettes per day (range, 5–35) with an estimated nicotine consumption of 23.2 mg/day (range, 0.6–56). The latter estimate is based on Federal Trade Commission smoking machine delivery data, with all the uncertainties that that method involves (Kozlowski *et al.*, 1980) and the number of cigarettes smoked each day. Several subjects drank alcohol (mean 13.9 g/week, range, 2.8–31.3); all but one used marijuana (range, one cigarette per month to four per day); two subjects used cocaine occasionally; and none used narcotics or sedative hypnotic drugs. Five additional men of similar age and smoking history served as control subjects for assessment of cardiovascular responses to nicotine. Under conditions similar to those described subsequently, the subjects received, after being told that nicotine or salt water, 0.9% sodium chloride, might be administered i.v. Results of biochemical tests of liver function

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ABBREVIATION: AUC, area under the concentration-time curve.

(serum glutamic-oxaloacetic transaminase, lactic dehydrogenase, alkaline phosphatase, total protein and albumin) and kidney function (blood urea nitrogen) were within normal limits in all subjects.

Experimental protocol. Subjects were studied in the morning after overnight abstinence from cigarette smoking. Ammonium chloride, 2 g four times each day, was administered the day before and during the study to acidify the urine and maximize renal elimination of nicotine and minimize variability among subjects due to differences in urinary pH. Subjects remained in the recumbent position, except to void, during the period of cardiovascular measurements. At least 1 hr before nicotine administration, i.v. catheters were inserted into an antecubital vein of one arm for injection of nicotine, or saline in the control subjects, and in a forearm vein of the other arm for blood sampling. Every 5 to 10 min, heart rate, blood pressure and temperature of the fingertip skin were recorded by electrocardiogram (Grass model 7 polygraph), Infrasonde (Marion Scientific Corporation, Costa Mesa, CA) and a thermocouple taped onto the index finger (Bailey Instruments, Saddlebrook, NJ), respectively. Cardiovascular measurements were recorded for 30 min before, during and for 90 min after cessation of nicotine or saline infusion.

Nicotine bitartrate was made up in sterile solution in 0.9% sodium chloride as described previously (Rosenberg *et al.*, 1980). Nicotine was infused at a constant rate by Harvard pump in doses of 1.0 (four subjects), 1.5 (five subjects) and 2.0 (five subjects) μg/kg/min for 30 min. Infusions were well tolerated by all subjects. Blood samples, 3 ml each, were collected for nicotine analysis before, 5, 10, 15, 20 and 30 min during and 5, 10, 20, 30, 60, 90, 120, 150, 180 and 210 min after the end of nicotine infusion. Urine was collected before and at 1- to 2-hr intervals for 8 hr after completion of nicotine infusion.

Nicotine and cotinine analysis. Plasma samples were assayed for nicotine and cotinine concentrations by gas chromatography using nitrogen-phosphorus detection (Jacob *et al.*, 1981). Sensitivity of this assay is 1 ng/ml with a coefficient of variation of 4.9 and 4.2% for nicotine and cotinine, respectively, at concentrations of 5 ng/ml.

Pharmacokinetic analysis. Terminal half-life and elimination rate constants were computed by linear regression of the log plasma nicotine concentration *vs.* time from 90 to 210 min. Plasma nicotine clearance was computed as dose/area under the nicotine plasma AUC. Total AUC was estimated using the trapezoidal rule with extrapolation of the terminal portion to infinity. Area contributed by the presence of nicotine before infusion was computed as C_0/K, where C_0 was the initial (preinfusion) concentration and K the terminal elimination rate constant, and was subtracted from total AUC to yield a net AUC, which was used to compute clearance. Renal clearance was computed as urinary excretion of nicotine/AUC plasma nicotine concentration, using urinary excretion and AUC for the time period during and up to 3 hr after the end of the infusion. Metabolic clearance was computed as systemic minus renal clearance. Steady-state volume of distribution was computed using the model-independent method of Benet and Galeazzi (1979), with appropriate correction for constant infusion dosing.

Protein binding studies. N-[*methyl*-^{14}C]-labeled *l*-nicotine-*d*-bitartrate (Amersham/Searle Corporation, Des Plaines, IL), with a purity of 95% as determined by thin-layer chromatography and a specific activity of 57 mCi/mmol, and unlabeled nicotine were added to fresh plasma obtained from three healthy men and three healthy women to achieve a final concentration of 10 to 180 ng/ml. One milliliter of plasma was dialyzed against 1 ml of 0.15 M phosphate buffer, pH 7.4, at 37°C for 16 hr. The unbound fraction of nicotine was calculated from the radioactivity measured in buffer and plasma at equilibrium. Comparisons of binding in serum *vs.* heparinized plasma revealed no difference; studies reported herein were performed using plasma.

Statistical analysis. Statistical analysis was performed by use of repeated measures analysis of variance with Newman-Keuls post tests, paired *t* tests and linear regression. The relationship between pharmacological responses and plasma concentration of nicotine was examined by plotting simultaneous values for the quantities against each other. The degree of hysteresis, that is, area included within the loop, was tested for significant difference from zero using the *t* test.

Results

Preinfusion plasma nicotine and cotinine concentrations averaged 3.5 ng/ml ± 3.5 (S.D.), range 0 to 11.4 ng/ml, and 111.8 ng/ml ± 63.8, range 8 to 234 ng/ml, respectively. Plasma concentrations of nicotine increased over the 30 min of infusion, declined rapidly immediately after the end of infusion and then in a slower log linear fashion from 90 to 210 min (fig. 1). As seen in table 1, total clearance averaged 1292 ± 293 ml/min and renal clearance averaged 203 ± 58 ml/min, accounting for 15.7% of total clearance. Terminal half-life averaged 119 ± 44 min. Considerable interindividual variability in both metabolic and renal clearance, as well as volume of distribution, were noted (fig. 2). Infusion rate had no influence on any of the pharmacokinetic parameters (table 1). There was no significant correlation between initial concentration of cotinine and metabolic clearance of nicotine.

The cardiovascular effects of the 2 μg/kg/min of nicotine infusion are illustrated in figure 3. Responses to other infusion rates were similar. Heart rate and blood pressure increased soon after onset of infusion, whereas skin temperature declined gradually during the course of infusion. During infusion of nicotine, mean heart rate increased 16.4 ± 9.1 beats/min ($P < .01$) and skin temperature decreased 5.8 ± 3.8°C ($P < .01$). Systolic blood pressure increased 8.8 mm Hg ± 5.4 compared to the preinfusion pressure ($P < .05$), but the overall systolic blood pressure in this small sample was not significantly different between nicotine and saline conditions. Diastolic blood pressure increased slightly and tended ($P < .10$) to be higher during nicotine infusion.

To visualize better the relationship between heart rate and skin temperature responses and plasma concentrations of nicotine within subjects, a hysteresis analysis was performed (fig. 4). This analysis shows that heart rate increased rapidly and reached a maximum by 5 min after the onset of nicotine infusion, thereafter remaining constant despite rising nicotine plasma concentrations. A given plasma concentration of nicotine was associated with a lower heart rate as nicotine concentrations declined compared with that which was observed while nicotine concentrations were increasing, consistent with the development of partial tolerance. The area enclosed within the

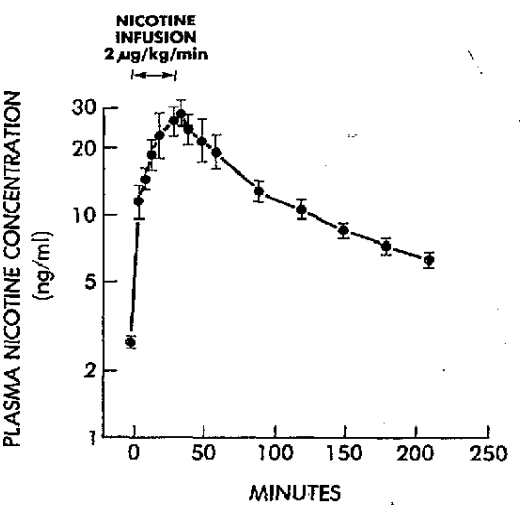

Fig. 1. Plasma nicotine concentrations (±S.E.M.) in five subjects during and after constant infusion for 30 min.

TABLE 1

Pharmacokinetics of nicotine, 30-min infusion

Patient	Body Wt.	Total Clearance	Renal Clearance	Nonrenal Clearance	V_{dss}[a]	$T_{1/2}$[b]
	kg	*ml/min*	*ml/min*	*ml/min*	*liters*	*min*
I. Low Dose (1 μg/kg/min)						
1	66.6	1281	139	1142	221	128
2	70.9	1897	268	1629	249	66
3	78.1	1045	220	825	96	74
4	60.8	886	150	726	136	125
Mean	69.1	1277	194	1083	176	98
S.D.	7.3	444	61	403	72	33
II. Medium Dose (1.5 μg/kg/min)						
1	96.1	1547	232	1315	117	107
2	87.0	760	233	527	222	239
3	66.4	1385	220	1165	186	94
4	65.0	1491	221	1270	204	151
5	64.3	1338	95	1243	201	117
Mean	75.8	1304	200	1104	186	142
S.D.	14.8	315	59	327	41	58
III. High Dose (2.0 μg/kg/min)						
1	56.8	1557	285	1272	154	74
2	78.8	1123	120	1003	154	105
3	63.0	1387	181	1206	145	96
4	62.5	1211	210	1001	241	151
5	90.0	1180	266	914	230	144
Mean	70.2	1291	212	1079	185	114
S.D.	13.7	178	66	152	46	33
Overall						
Mean	71.8	1292	203	1089	183	119
S.D.	12.1	293	58	279	49	44
CV[c] (%)		22.7	28.6	25.6	26.6	37.2

[a] V_{dss} = steady state volume of distribution.
[b] $T_{1/2}$ = terminal half-life.
[c] CV = coefficient of variation.

heart rate hysteresis loop was significantly greater than zero ($P < .01$). In contrast, the changes in skin temperature paralleled blood concentrations of nicotine while increasing or decreasing. The area within the skin temperature hysteresis loop was not significantly different from zero.

To examine the relationship between plasma concentrations of nicotine and cardiovascular responses across subjects, cor-

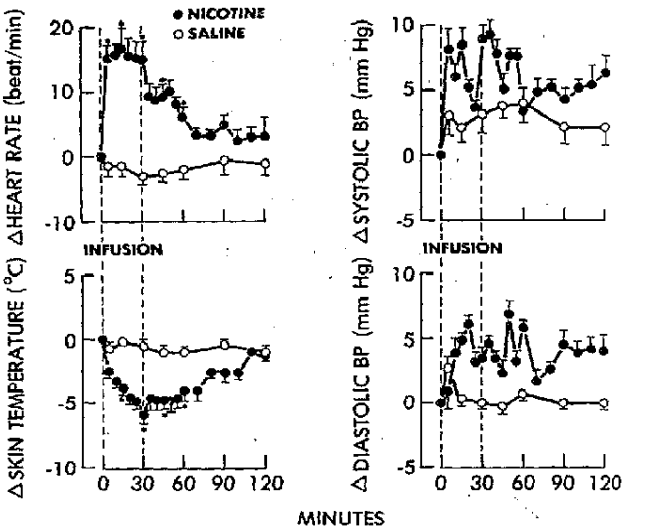

Fig. 3. Cardiovascular responses to nicotine ($N = 5$) and saline ($N = 5$) infusions. Asterisks indicate $P < .05$, comparing nicotine and saline conditions by Newman-Keuls post-test. BP, blood pressure.

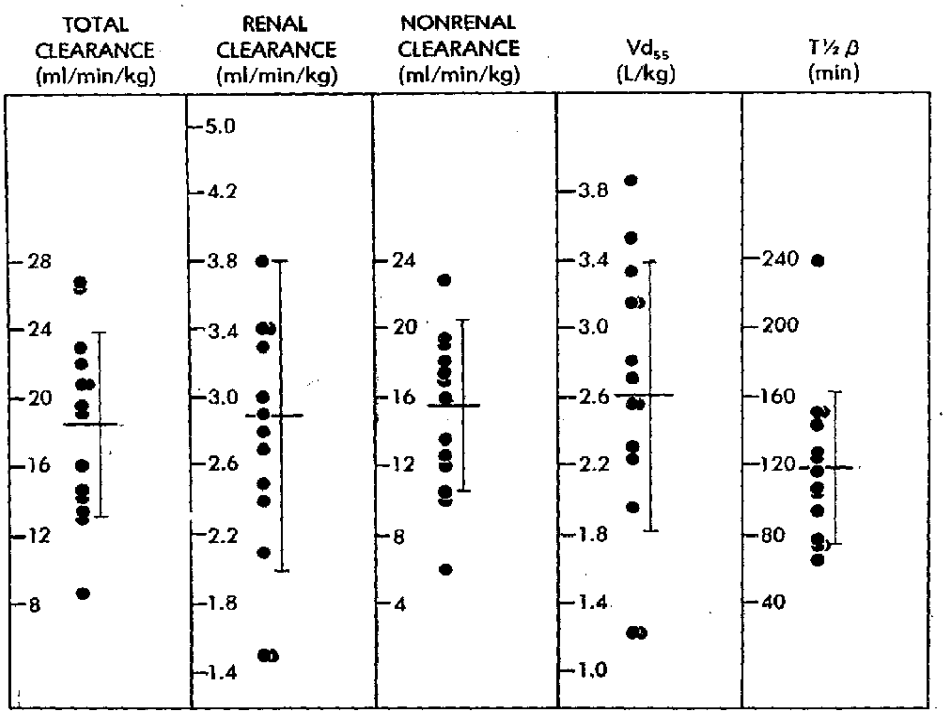

Fig. 2. Interindividual variability in pharmacokinetics of nicotine in 14 healthy male cigarette smokers. V_{dss} = steady-state volume of distribution. $T_{1/2\beta}$ = terminal half-life.

relations between maximal increase in plasma nicotine from base line and maximal cardiovascular responses were examined (table 2). The magnitude of effects was quite variable across subjects and no significant relationships between change in plasma concentrations of nicotine and cardiovascular responses were observed.

Plasma protein binding of nicotine averaged 4.9% (±2.8) and was no different as a function of nicotine concentration or sex of the subject.

Discussion

Conclusions from our earlier study of nicotine disposition kinetics after repeated bolus injections (Rosenberg *et al.*, 1980) were limited because blood concentrations of nicotine increased rapidly during and declined rapidly after injection. These changes produced errors in computing AUC and, therefore, clearance. The pharmacokinetic parameters differed with duration of nicotine exposure, probably because blood concentrations of nicotine had not been measured long enough to determine a true half-life. In the present study, using constant nicotine infusions and measuring blood concentrations of nicotine for 3 hr after the end of the infusion, we are confident of our estimates of clearance and terminal half-life.

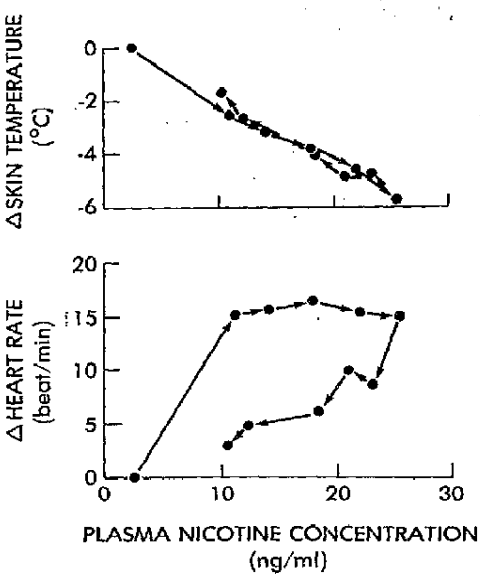

Fig. 4. Hysteresis loops for skin temperature and heart rate responses plotted against simultaneous plasma nicotine concentration (mean values $N = 5$). Arrows indicate progression of time during and after nicotine infusion.

The metabolic clearance of nicotine averaged 1085 ml/min, a value higher than previously reported in similar subjects (Rosenberg *et al.*, 1980). Nicotine is primarily metabolized by the liver, although some is metabolized by the lung (Turner *et al.*, 1975). Assuming mostly hepatic metabolism, the metabolic clearance of nicotine is approximately 70% of hepatic blood flow, consistent with a high extraction from the hepatic circulation. Marked interindividual variability in the rate of nicotine metabolism was observed, as is the case for rapidly metabolized drugs.

Cigarette smoking accelerates the metabolism of many drugs including nicotine (Jusko, 1978; Beckett *et al.*, 1971). The degree of acceleration cannot be predicted from the number of cigarettes reported smoked. Plasma cotinine, the major metabolite of nicotine, is potentially a marker of nicotine consumption (Gorrod and Jenner, 1975; Langone *et al.*, 1973). It might be hypothesized that cotinine would be a better indicator of tobacco smoke exposure and effects on drug metabolism. However, we found no relationship between cotinine concentration and metabolic clearance of nicotine. In studies with rodents, nicotine metabolic clearance appears dose-dependent (Miller *et al.*, 1977). However, our data in humans give no evidence of dose dependence.

Renal clearance, in acid urine, averaged 203 ml/min, representing 16% of total clearance. The observation that nicotine renal clearance is much higher than glomerular filtration rate indicates that there is net secretion of nicotine into acid urine.

Volume of distribution of nicotine was quite large and variable from person to person. Variability in volume of distribution cannot be explained on the basis of variable protein binding, because the latter is so low it is inconsequential. The large volume of distribution accounts for the relatively long terminal half-life in the presence of rapid metabolism. The nearly 2 hr half-life of nicotine is consistent with observations that blood concentrations of nicotine accumulate for 4 to 6 hr with regular smoking and that substantial concentrations of nicotine persist in the blood overnight after a day of smoking (Benowitz *et al.*, 1981).

The up to 3-fold interindividual variability in rate of nicotine metabolism could be a significant determinant of smoking behavior. Variable metabolism means that even if different smok-

TABLE 2

Increase in plasma concentrations of nicotine and maximal cardiovascular responses

Correlation coefficient comparing ΔPNC and increased response across all subjects. Data are expressed as mean ± S.D.

Infusion Rate	No. of Subjects	ΔPNC[a]	ΔHR[b]	ΔSBP[b]	ΔDBP[b]	ΔST[b]
ug/kg/min		ng/ml	beats/min	mmHg	mmHg	°C
1.0	4	13.2 ± 5.6	12 ± 6	10 ± 3	8 ± 4	−5.6 ± 1.2
1.5	5	29.6 ± 15.6	20 ± 2	9 ± 7	7 ± 5	−2.6 ± 1.9
2.0	5	27.0 ± 3.5	21 ± 7	13 ± 6	7 ± 3	−6.7 ± 3.8
All	14	24.0 ± 11.8	18 ± 6	11 ± 5	7 ± 4	−5.0 ± 3.0
r			0.22 (N.S)	0.31 (N.S.)	0.03 (N.S.)	−0.30 (N.S.)

[a] ΔPNC = Maximal increase in plasma nicotine from preinfusion concentration.
[b] ΔHR, ΔSBP, ΔDBP, ΔST = maximal change in heart rate, systolic blood pressure, diastolic blood pressure and skin temperature, respectively, during nicotine infusion.

ers inhaled the same quantity of nicotine, there will be substantial differences in average blood concentrations of nicotine. This may contribute to the poor correlation between a cigarette's nicotine delivery and blood concentrations (Russell *et al.*, 1980). Slow metabolizers might be at higher risk for nicotine-related adverse health effects. Rapid metabolizers might smoke more cigarettes to maintain body nicotine levels and thus inhale relatively more potentially toxic products, such as carbon monoxide and tar.

Nicotine infusions achieved plasma concentrations and cardiovascular effects similar to cigarette smoking (Russell, 1976). Response patterns were different for heart rate and skin temperature. Heart rate increased after low concentrations of nicotine and reached a plateau, beyond which increasing blood concentrations of nicotine had no effect. As blood concentrations declined, heart rate declined, but with evidence of tolerance when compared with the response to the ascending nicotine concentrations.

The heart rate increase occurred soon after nicotine exposure, at a time when subjects reported a sense of arousal. Cryer and co-workers (1976) reported that heart rate increased before there was an increase in circulating catecholamines. It is likely that heart rate acceleration is part of a general central nervous system-mediated sympathetic neural arousal. Rapid disappearance of subjective arousal and evidence of tolerance to heart rate effect are consistent with central nervous system adaptation observed for many psychoactive drugs (Kalant *et al.*, 1971).

Cigarette smoking and nicotine ingestion are known to constrict cutaneous blood vessels and decrease blood flow to hands and feet (Roth *et al.*, 1944; Eckstein *et al.*, 1957). Skin temperature is related to cutaneous blood flow. Fingertip and toe temperatures consistently fall after nicotine exposure. We found that, in contrast to the heart rate response, the reduction in skin temperature was progressive during nicotine infusion. The magnitude of the response was directly related to the blood concentration of nicotine during and after nicotine infusion. There was no evidence of tolerance.

In addition to its central nervous system effects, nicotine directly releases norepinephrine from vascular nerve endings (Burn, 1960). Nicotine inhibits the release of prostacyclin, a vasodilating prostaglandin, from vascular tissue (Sonnenfeld and Wennmalm, 1980). These actions could result in vasoconstriction. Local blood vessel effects of nicotine might explain a correlation between magnitude of cutaneous vasoconstriction, as indicated by reduced skin temperature, and circulating nicotine levels. Studies directly measuring response to nicotine are needed to test this hypothesis.

Our observations have potential clinical importance. That low concentrations of nicotine increase heart rate to a maximum suggests the heart rate response to cigarette smoking, after the day's first few cigarettes, will not vary with amount of nicotine delivered, a finding, in fact, observed (Benowitz *et al.*, 1981). The possibility that vasoconstriction is more related to blood concentrations of nicotine, at least within a particular individual, suggests that the extent and duration of vascular responses might be sensitive to the amount of nicotine consumed and persist as long as nicotine circulates. Indeed, cutaneous vasoconstriction, as assessed by thermography of the hands and feet, lasts as long as 90 min after a single cigarette (Gershon-Cohen *et al.*, 1969). We would predict that vasoconstrictor effects would persist in cigarette smokers overnight because nicotine remains in the circulation over that period of time.

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